STERILIZATION.

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A knowledge of sterilization is becoming more and more important to the pharmaceutical profession as the administration of medicines by the sub-cutaneous and intravenous routes increases in popularity.

The purpose of sterilization in pharmacy is to destroy such forms of microbic life as may exist in medicinal preparations and the various containers, etc., used in handling and dispensing them. Of the innumerable living things that may be found in crude drugs, only the microscopic forms, such as yeasts, molds and bacteria usually escape elimination in the course of manufacture. It is necessary to destroy molds and yeasts, because their growth may result in fermentation or other forms of decomposition, with deterioration, and the production of harmful by-products; but far greater in importance, are the bacteria, which have an additional property only rarely exhibited by yeasts and molds—that of multiplying in the animal body and causing disease.

These microscopic organisms are ubiquitous in nature; one can think of few places where they may not be found. Some of them will grow only upon living tissues (parasites), others will grow only upon dead matter (saprophytes). There are a few species which seem able to multiply even in distilled water, depending upon gases absorbed from the air for their nourishment. Some of the bacteria die quickly when removed from media favorable to their growth, others are so tenacious of life that they may exist for years and possibly for centuries in unfavorable surroundings.

In considering the problems of sterilization it is these latter bacteria to which we are compelled to give especial attention. When organisms of this class find themselves in a medium unsuited to their rapid growth, they go into a state that may be considered a kind of hibernation. The vital part of the bacterial cell is collected within a very resistant capsule, which, with its contents, is known as a spore. The spore state is not a means of reproduction in the sense of multiplication, it is merely a means of self-preservation. One bacillus makes one spore, and one spore makes one bacillus. Therefore, while spores are like seeds in preserving the essential vitality of the cell, they differ in that seed formation is chiefly a reproductive (multiplying) function.

Some spores are able to withstand heat to the boiling point of water for several hours, while bacteria not in the spore state are killed when heated to 60° C. for less than one hour. This difference in the degrees of heat required for killing spores and bacteria, also holds with regard to other germicidal influences. Spores are far more resistant, to phenol for instance, than are non-sporogenic bacteria. The state of the preparation to be sterilized with regard to moisture, is of great importance, since bacteria which are entirely dry may be heated to very far beyond the boiling point of water without killing them, an important point in discussing methods for sterilizing tablets and powders.

Fortunately there are few common sporogenic bacteria which cause disease if they gain entrance to the uninjured intestinal tract.

Whether a remedy is to be administered upon the unbroken skin or mucous membranes, or enterally, that is by mouth or by rectum, or parenterally, under the skin or into a vein—these differences are of fundamental importance. For local application or enteral administration, approximate sterility is usually sufficient, but for parenteral injection or for use in a surgical operation, complete asepsis is requisite. For instance, the tetanus bacillus taken by mouth, is not likely to do any harm, but injected beneath the skin, will, under favorable circumstances, cause tetanus or lock-jaw. By the term parenteral injection is meant, as I have just indicated, the internal administration of a remedy by other than the gastro-intestinal route, that is sub-cutaneously, intravenously, intraperitoneally, intramuscularly, intraspinally, etc.

Unless remedies intended for local or enteral administration are suspected to contain disease-producing bacteria, sterilization is not generally thought of in connection with them, except in so far as it concerns their preservation. For this purpose the addition of chemical substances is simpler, and since their action is continuous they are more efficient than heat or filtration. This is well exemplified by the fact that, with few exceptions, pharmaceutical preparations, e. g., tinctures, fluid extracts, syrups, etc., contain sufficient alcohol, sugar or other preservative to prevent decomposition through bacterial growth. It is thus apparent that the chief concern we have with sterilization is the preparation of remedies intended for parenternal use.

By sterilization we mean the destruction or removal of every form of reproductive life. To accomplish this we have several methods, physical, chemical and mechanical. The physical methods are heat, electricity and light,—sunlight, ultra-violet rays, etc.

Chemical sterilization is accomplished by the use of such substances as phenol, corrosive sublimate, etc.

The mechanical methods for sterilization of greatest service are filtration, and centrifugation.

Heat. The *naked flame* may be used for sterilizing certain instruments, platinum vessels, spatulas, and small pieces of glassware. A Bunsen burner is generally used for this purpose.

Dry heat may be used for the sterilization of glass bottles, and other glass vessels of various kinds, for powders that are not injured by a high degree of heat, and, where an autoclave is not available, for the sterilization of cotton. It cannot be used for organic or volatile substances or for aqueous solutions. To prepare them for sterilization, bottles and flasks are stoppered with cotton and, as an extra precaution, they may have a piece of paper tied over their mouths before being placed in the oven. They must be perfectly dry. Other pieces of glassware may be wrapped in filter paper or in ordinary Manila paper.

The best arrangement for sterilizing by dry heat is the dry-wall sterilizer of the Lautenschlager pattern. This is a special laboratory apparatus heated by gas, with the walls so arranged that the hot air is kept in motion and approximately, the same heat is maintained in all parts of the oven. A small dry-wall sterilizer is not expensive, but, since the desired heat may be obtained in an ordinary bake-oven, it is likely that in pharmacies, where a great deal of work requiring sterilization is not done, the pharmacist will use the kitchen range, and

he may do so with perfect safety. For small pieces of glassware it is the rule to consider sterilization accomplished when the paper wrapping has started to char. A precaution that must be noted, however is, that, if the oven is extremely hot, the paper may char almost immediately, before the heat has had time to penetrate to the vessel. In using the dry-wall sterilizer, it is customary to run the heat up to 180° C., and continue it at this point for two hours.

Steam and boiling: The method which has by far the widest field of application is sterilization by steam. There are two methods: (1) by steam under pressure in a closed chamber called an autoclave, (2) by a double walled hood, in which materials are subjected to streaming steam without pressure.

Steam has much greater penetrating power than dry air. Bacteria entirely dry are sometimes not killed even by heating them to a temperature of 150° C., while all bacteria are destroyed in steam under pressure at 120° C. The best apparatus for sterilizing in streaming steam is the Arnold sterilizer. This consists of a pan with a thin false bottom into which water flows and comes in contact with the heat in such a thin layer that it is almost immediately converted into steam. As the steam passes up through the central opening, water trickles down through small holes at the corners of the pan. The sterilizing chamber has a double wall, which helps to conserve the heat. The inside chamber is maintained at a temperature of about 100° C. for at least twenty minutes after the materials being sterilized have reached this temperature. Such a temperature is not sufficient to kill spores and in order to destroy them the "Tyndall," or discontinuous method, must be used. That is to say, substances to be sterilized by streaming steam are heated for twenty minutes on three successive days. The idea is that all active bacteria are killed on the first day; during the period after heating, until the next day, if there is any nourishment present, the spores develop into bacteria and are killed at the second heating. Any spores that may have escaped the second heating are pretty certain to have developed by and will be killed on the third day. While this is the explanation given by some writers, others think that the spores are gradually weakened by the repeated heating, until they are finally destroyed completely on the third day. Fluids that will not stand the temperature of the autoclave, but which will stand boiling may be efficiently sterilized in the Arnold sterilizer by the discontinuous method, provided they contain sufficient nutrient material to favor the development of any possible spores, otherwise the method may fail and filtration, with or without the use of an antiseptic, must be employed. The pharmacist who does not have this special apparatus, can secure the same result if the bottles or flasks containing the fluid to be sterilized are placed in water in a covered vessel, such as a wash boiler or a covered saucepan. The vessels containing the fluid to be sterilized should be raised from the bottom of the pot by a piece of woven wire or something of the kind, and their tops should be well protected so that the cotton stoppers will not get wet.

A still simpler method is to place the fluid in an Erlenmeyer or Florentine flask, which has been sterilized by dry heat, and boil it for fifteen to twenty minutes over the naked flame on three successive days. This method is open to the objection that there is considerable evaporation and therefore it cannot be used if the original bulk of the fluid must be maintained. If there is no objection to it,

a surface coating of liquid paraffin will prevent evaporation, unless the boiling is too vigorous.

It is well known that the boiling point of mineral oils is much greater than the boiling point of water. These may be sterilized by heating them over the naked flame, to 160° C., in a suitable flask.

Steam, under pressure in a closed chamber, known as an autoclave, is the most useful means for sterilizing the ordinary culture media employed in bacteriological laboratories. This is the best method for sterilizing dressings, physiological saline solution, and other fluids not injured by the autoclave temperature. For sterilization in the autoclave, the temperature is maintained at 115° C. to 120° C. under a pressure of from ten to fifteen pounds for twenty to forty minutes.

Fluids not intended for parenteral administration, such as foods of various kinds, that may be contaminated by disease-producing bacteria, are efficiently sterilized by Pasteurization. This is accomplished by heating to a temperature of at least 60° C. maintained for one-half to one hour, the time depending upon the degree of heat used. A temperature so low as 56° C. is sufficient to kill many non-sporogenic bacteria and substances destroyed by a degree of heat greater than this, may usually be sterilized by subjecting them to this temperature, for about one-half hour, from five to seven successive days. In some laboratories the blood serum mixture used for the diagnosis of diphtheria is sterilized by this method.

Sterilization of Fluids in Sealed Capsules: It is possible for the pharmacist to dispense solutions efficiently sterilized, without the use of any apparatus other than a vessel that may be used as a water bath. The solutions that are to be dispensed, however, must be placed in glass containers hermetically sealed—the most useful form being the bottle with a long narrow neck commonly called an ampoule. These, stoppered with cotton and wrapped in paper, or placed in a metal box, are sterilized by dry heat, maintained at 180° C. for two hours. After the fluid to be dispensed is filled into them, through a narrow tube or by vacuum, the necks of the ampoules are sealed in a blow-pipe flame. They may then be sterilized at any temperature their contents will withstand. For instance if it is a question of a cocain solution, the ampoules may be heated to about 60° C. for one hour on seven successive days, at the end of which time they should be sterile—cocain hydrochlorid is said to withstand a temperature of 70° C. without decomposing. If the substance in solution will withstand 100° C., the ampoules may be boiled for one-half to one hour on three successive days. Should a higher temperature be unobjectionable, the autoclave conditions may be obtained by the use of a strong solution of calcium chlorid or other salt in the water bath or one may use a mineral oil. If liquid paraffin is used the ampoules are easily cleansed after sterilization by rinsing them in ether.

Chemical Methods: The chemical methods for sterilization include the use of the well-known germicides, such as bichloride and oxycyanide of mercury, carbolic acid, liquor cresolis comp., formaldehyde, etc. There are times when solutions of these may be used in such strength that they will efficiently sterilize the substances in question. In by far the greater majority of instances, however, they can be added only in antiseptic strength, thus being used merely as preservatives. Cresol, the substance employed at present in practically all laboratories

as a preservative for biological materia medica, is used in various strengths ranging from 0.2 of 1 percent to 0.5 of 1 per cent combined with filtration for serums. In this strength it is only antiseptic. It does not become germicidal until we reach the minimum concentration in water of 2 percent, and even then considerable time must be allowed for its action upon spores. The sterilization of glass vessels, by allowing a strong disinfectant to remain in them cannot be recommended. Tiny scratches and fractures may still be large enough to protect bacteria, since capillary attraction might prevent the solution from coming in contact with them.

There are many well-known substances which are practically without germicidal value but which are efficient as antiseptics.These include boric and salicylic acids, camphor, thymol, etc. Glycerin in high concentration is quite efficient. Sugar is an efficient preserative and is widely used as such, especially in preserved foods.

Filtration: Properly used, and with an appreciation of its limitations, filtration is a method for sterilizing which may be of great value to the pharmacist, for the reason that by this means solutions may be freed from bacteria without the slightest change in their chemical composition. Filtration, of course, cannot be used for thick or syrupy liquids, nor can it be used for suspensions or emulsions of any kind. Many ingenious arrangements have been devised for the filtration of solutions required to be sterile. Some of these combine, in the apparatus, both filtering and filling devices.

Contamination After Sterilization: After a piece of apparatus or a preparation has been efficiently sterilized, proper precaution must be taken to prevent subsequent contamination. There are bacteria not only on *every object* with which it may come in contact, but also in the air. Consequently, if it be left uncovered, except under special conditions, contamination is almost certain to result. When fluids are transferred from one bottle or flask to another, the exposed part of the lip over which the fluid will run, must first be sterilized in the Bunsen flame. Such operations must be done only in specially constructed and protected rooms or under sterile or dust-proof hoods or other covers.

When a sterile fluid has been obtained in bulk, transferring it into smaller containers is one of the interesting problems confronting those responsible for the preparation of antitoxins and vaccines. Such operations must be carried on in specially prepared rooms or under dust-proof hoods. An ingenious and convenient substitute is the oven, as suggested by Professor Cook, or one may use a box lined with blotting paper saturated with a disinfectant solution. If one is expert, the transference may be made by pouring or by means of a sterile bulb pipette. These procedures are suitable for small quantities but for large amounts it is customary to use a graduated burette or a special contrivance, of which there are many on the market. In my experience a properly arranged burette is the most satisfactory filling apparatus for general purposes. In one of the larger manufacturing biological laboratories the filling of serums and vaccines is done in special rooms which are furnished with washed and filtered air. The rooms are disinfected with formaldehyde at least once a week and the operatives wear sterilized gowns and caps.

Tests for Sterility: In spite of the most approved *technique* and the most efficient apparatus, it may never be taken for granted that a fluid has been freed from living bacteria and has remained sterile throughout the process without proper bacteriologic controls. These controls must be such that they will meet every peculiar condition likely to influence the growth of bacteria in the solution. We read frequently of persons who make control cultures by placing a drop of the fluid upon nutrient agar—a solid culture medium. Sometimes fluids tested in this way, contain sufficient antiseptic to prevent the growth of bacteria which are present. This method is therefore faulty. Control tests must be made in a fluid medium, and the bulk of fluid must be sufficient to dilute the antiseptic contained in the substance under examination to such an extent that it can have no possible antiseptic power.

The contaminating bacterium may be an aerobe (i. e., one that requires oxygen for its growth) or it may be an anaerobe (one that requires the absence of oxygen). The control culture must afford conditions for the growth of bacteria of both kinds or two sets of controls must be made. Fortunately the latter is not necessary because the common fermentation tube affords conditions suitable for the growth of both varieties. The necessary condition is that the *bouillon* must have been freshly sterilized in order to drive off suspended or dissolved oxygen since this would prevent the growth of anerobic bacteria. After shaking the bottle containing the substance to be tested, to be sure that any contained bacteria are distributed throughout, a small quantity of the fluid is withdrawn with a sterile pipette and transferred to the freshly sterilized fermentation tube. Bacteria present may be slow growers and their presence may not be recognized even after twenty-four hours incubation. The reading should not, therefore, be taken earlier than forty-eight hours after the control culture has been made. Bacterial growth of any kind should be sufficient to cause the material to be withheld.

If the substance under examination is one that may possibly be contaminated by the tetanus bacillus, that is to say, if it is a nutrient fluid or a biological product that has been developed upon nutrient media, or if it is the serum of an animal which might have had tetanus toxin in its blood at the time of bleeding or if in any other way toxic or harmful substances might have gained entrance to it, a cultural test is not sufficient. A quantity, at least relatively equal to the maximum dose that may be used for the patient, should be injected into an animal and the effects of the injection on the animal observed for a sufficient length of time to detect the results of a possible harmful contamination.

Those who have had much experience in the preparation of substances intended for subcutaneous administration, are familiar with the many sources of danger and know of so many instances of serious errors that they are inclined to make too many controls rather than too few. In the larger biological laboratories it is customary to have each product tested in bulk, by two bacteriologists working independently. Products that have passed this test are filled into proper receptacles by means of a carefully sterilized burette or other filling device. Cultural tests are made of the filling device before any filling is done, and a number of filled containers selected at random are examined bacteriologically to be certain the filling has been done properly. In addition to this the special rooms above mentioned are set aside for the filling, and these are given unusual care.

It is evident that if remedies intended for parenteral administration are to be dispensed only after passing through the rigorous tests mentioned above, they must be prepared by persons who have had training in bacteriological *technique*. A realization of this fact is leading the colleges of pharmacy to give their students advanced training in special bacteriological *technique*. To be useful such courses should be thorough. It is apparent that here a little knowledge might be a dangerous thing indeed. Certain criticisms of the tests suggested for the preparation of remedies for parenteral administration will undoubtedly be made. Some persons will say, many others will think, that we have been getting along all these years with much simpler methods—why not continue? The reason we may not continue with them is that these simpler methods are the cause of serious and even fatal infections. We learn of some of them, there are probably ten times as many that we never hear about. The demand for sterile solutions for hypodermic and intravenous injection is destined to increase, these solutions must be sterile and their sterility must, before use, be demonstrated by adequate tests.

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DISCUSSION.

M. I. Wilbert, of Washington, D. C., said that, from a pharmaceutical point of view, he believed Dr. Hitchens had demonstrated clearly and fully that the only safe and reliable method of sterilization was the very simple one outlined in the German Pharmacopœia: "Sterilization should be done in accordance with established bacteriological *technique*." That was the only safe method of sterilization. It was absolutely unsafe to follow any published prescription or formula. Discontinued sterilization was often spoken of as being absolutely safe, but it was not. In connection with solutions that were themselves antiseptic, like physiological salt solution, if the substance were contaminated by spore-bearing organisms, it could be sterilized half a dozen times, and still be extremely dangerous. Discontinued sterilization depended largely on the development of spores into viable organisms, recognizing that spores themselves are not affected at the ordinary temperatures used. Therefore, ordinary sterilization, unless at very high temperature, would have no effect. Sterilization at high temperatures was not without possible dangers. Repeated work on distilled water had shown that this form of water afforded favorable conditions for the growth of microorganisms; and under certain conditions, as had been shown recently, distilled water might contain, after three or four days, a tremendous number of organisms. The use of such a contaminated water is objectionable from every point of view, even if properly sterilized it would involve risks from generated poisons or the killed organisms themselves.

In connection with the possibilities of the sterilization of local anesthetics, a considerable amount of work had been done in Germany; but the field was so large that there was no end to the possibilities in that direction. Cocaine solutions, that Dr. Hitchens had said could be sterilized at 70 degrees, could also be heated to a considerably higher temperature for a very short time. So that with recently distilled water, and satisfactory conditions so far as containers were concerned, solution of cocaine could be heated to even 100 degrees for a few minutes. Such a solution would be reasonably safe; not absolutely safe, but reasonably so. He said Dr. Hitchens had covered so much ground that it was hard to grasp it all at once, but he hoped the members would fully appreciate his first statement, which was that sterilization should only be successfully done with adequate control, and in strict accordance with bacteriological *technique*.

Chairman Havenhill commented that this certainly was a live subject, or soon would be, with pharmacists. He said he understood the next Pharmacopœia would contain a chapter on sterilization.

E. Fullerton Cook, of Philadelphia, said that one point Dr. Hitchens brought out was worthy of still further emphasis, viz.: that suggestion which made it possible for the phar-

macist to prepare sterile solutions in ampoules with the use of simple apparatus only. He suggests that suitable solutions in non-soluble glass ampoules, may be sterilized after the ampoules are sealed, by placing them in oil or saturated salt solution, and heating for fifteen minutes at a temperature of 115° C. This operation requires only the use of a covered vessel and a thermometer, while the results obtained would approximate those secured through the use of an expensive autoclave; that is, the temperature would be the same as that of the autoclave and the pressure, consequently, inside of each ampoule would be equivalent to the pressure in the autoclave under those conditions.

Such a process is not applicable to all substances, since the temperature required is prohibitive at times, and yet this plan is so simple and where it can be applied so easily and promises such satisfactory results, that it should not be passed over without the emphasis here given.

F. W. Nitardy, of Denver, said his practice was to use steam at 30 pounds pressure in the process of sterilization, keeping it at that point for fifteen minutes. He considered this sufficient sterilization.

Doctor Hitchens, responding to the last speaker, said he thought there was no doubt about the efficiency of this method, provided precautions were taken to have the steam completely replace the air in the chamber. And yet, without control, it would fail. In the ten years he had been doing this work, he had failed many times; but the failure was because somebody had not taken the precaution to remove the air, with the result that the sterilization would not be complete, unless the temperature was raised very high. As he had tried to bring out, and as Mr. Wilbert had kindly emphasized, sterilization must always be controlled by proper cultures. Such cultures are usually made in fermentation-tubes, containing freshly sterilized 2 percent dextrose *bouillon*. With all the precautions ordinarily taken, failure sometimes occurred.

Otto Raubenheimer, of Brooklyn, said he desired to emphasize one point—a thing that should be well known to pharmacists at this time; that distilled water was not necessarily sterile water. Some gentlemen seemed to be of the opinion that sterilization can be attained very easily. But Dr. Hitchens and Mr. Wilbert had properly emphasized the necessity of bacteriological control. He related his observance of a city milk-inspector taking samples of milk for analysis from several milk-wagons, and of the indifference to the exposure of these samples to contamination by bacteria. By the methods pursued by this inspector it must follow that the milk would show excess of bacteria, and he presumed that the milkmen from whom the samples were taken would be disciplined. This incident, he said, would show how carelessly such work is done,—work that should be done with great care and precaution; and all work of sterilization should be guarded with the utmost precaution.

METAL COLLOIDS—THEIR INCREASING IMPORTANCE AS REMEDIAL AGENTS.

CHAS. E. VANDERKLEED AND FRITZ HEIDELBERG.

Perhaps no field of chemical and physical research so well shows how rapidly progress is being made, as a glance at some recent developments in the study of colloids, and particularly at the changes that are being made in our conception of colloids. Just as we no longer believe that atoms are actually indivisible—though for all or nearly all of the purposes of the art of chemistry we may still so consider them; just as we no longer are convinced of the absolute indestructibility of matter—though for all practical purposes in chemistry and mechanics we may so calculate; so do we no longer adhere to Graham's original classification of matter into crystalloids and colloids. We may no longer look upon colloids as a distinct class of substances, separate and apart from other classes